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Title: Application of Ozone for Inactivation of *Escherichia coli* O157:H7 on Inoculated Alfalfa Sprouts

Author(s): R.R. Sharma, and A. Demirci, L.R. Beuchat, and W.F. Fett

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APPLICATION OF OZONE FOR INACTIVATION OF *ESCHERICHIA COLI* O157:H7 ON INOCULATED ALFALFA SPROUTS[1]

RATNA R. SHARMA[2] and ALI DEMIRCI[2,3,4]

[2]Department of Agricultural and Biological Engineering
[3]Life Sciences Consortium
The Pennsylvania State University
University Park, Pennsylvania 16802

LARRY R. BEUCHAT

Center for Food Safety and Department of Food Science and Technology
University of Georgia
1109 Experiment Street
Griffin, Georgia 30223-1797

AND

WILLIAM F. FETT

United States Department of Agriculture
Agricultural Research Service
Eastern Regional Research Center
Food Safety Intervention Technologies
600 E. Mermaid Lane
Wyndmoor, Pennsylvania 19038

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ABSTRACT

Chemical treatments to eliminate pathogens on inoculated seed sprouts have shown little success. This study investigated the effectiveness of aqueous ozone in killing Escherichia coli *O157:H7 on alfalfa sprouts. Alfalfa sprouts inoculated with a five strain cocktail of* E. coli *O157:H7 were immersed in water containing 21 ppm ozone for 2, 4, 8, 16, 32, 64 min at 4C. To increase availability and accessibility of ozone into sprout crevices, alternative treatments with continuous*

[1] Disclaimer: Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[4] Author for correspondence: TEL: (814) 863-1098; FAX: (814) 863-1031; E-mail: demirci@psu.edu

ozone sparging with and without pressurization were evaluated. Ozone treated sprouts were subjected to 12 psi hydrostatic pressure with pressurized ozone gas for 5 min to aid introduction of aqueous ozone into areas where pathogens may be hidden. Immersion of inoculated alfalfa sprouts (~10^7 CFU/g) in water containing 21 ppm ozone reduced the population of E. coli *O157:H7 by 0.85 log_{10} CFU/g after 64 min. There were no significant differences in log_{10} reductions between the ozone treatments and control or between treatment times. With the exception of 2 min treatments, continuous ozone sparging resulted in reductions of 0.83 - 2.20 log_{10} CFU/g, significantly higher (P $\leq$ 0.05) than reductions obtained by sparging with air (0.83 - 1.29 log_{10} CFU/g). Application of low hydrostatic pressure (12 psi) for 5 min subsequent to continuous ozone sparging for 2 to 64 min reduced* E. coli *O157:H7 populations by 2 log_{10} CFU/g. Reductions resulting from pressurized ozone treatments did not differ significantly from those resulting from unpressurized ozone treatments, except at 32 min. Ozone treatments did not exhibit a visible detrimental effect on sprout quality. Further investigation is required to develop methods applying ozone for the purpose of decontaminating sprouts and reducing health risks. Ozone exhibits the potential for successful post harvest treatment of sprouts and also to replace other chemical treatments currently being used in the seed sprout industry.*

INTRODUCTION

After being recognized as a human pathogen in 1982, *Escherichia coli* O157:H7 was implicated in outbreaks of infections associated with several different foods, including seed sprouts (Breuer *et al.* 2001). There were numerous foodborne disease outbreaks worldwide due to contamination of alfalfa, clover and mung bean sprouts with *E. coli* O157:H7 and *Salmonella* (Mahon *et al.* 1997; Mohle-Boetani *et al.* 2001; NACMCF 1999b). Sprouts are identified as a special concern, owing to their potential for supporting growth of pathogens during production (NACMCF 1999a; Prokopowich and Blank 1991). The sprouting process provides a warm and moist environment favorable to the growth of pathogens. In addition, tissue fluids from the sprouting seeds contribute nutrients for growth of microorganisms (Stewart *et al.* 2001; Taormina and Beuchat 1999a). Hara-Kudo *et al.* (1997) observed that the cotyledons and hypocotyls of radish sprouts become highly contaminated with *E. coli* O157:H7 when grown from seeds contaminated with *E. coli* O157:H7. Sprouts are generally consumed raw or slightly cooked and this further increases the probability of survival of pathogens and occurrence of outbreaks.

Contaminated seeds are the most likely source of pathogens in outbreaks associated with sprouts. The potential sources for seed contamination include irrigation water, use of inadequately treated manure as fertilizer, location of fields close to animal rearing facilities, and poor worker hygiene (NACMCF 1999b). Researchers are developing methods to eliminate pathogens on seeds without reducing germination percentage (Lang *et al.* 2000; Taormina and Beuchat 1999a, b). Decontamination of seeds by treating with 20,000 ppm of chlorine (using calcium hypochlorite) is recommended by the U.S. FDA (Federal Register 1999). However, in some cases, this treatment does not eliminate *E. coli* O157:H7 from laboratory inoculated seeds (Taormina and Beuchat 1999a) as evidenced by subsequent growth during sprouting. In addition, organic farmers who constitute about half the sprout growers are reluctant to use this decontamination method (EH update 1999).

If the pathogens are not completely eliminated from seeds or if contamination occurs during sprouting, pathogen populations may reach significant levels in mature sprouts (Stewart *et al.* 2001). Castro-Rosas and Escartin (2000) studied the ability of *Vibrio cholerae* O1, *Salmonella* Typhi, and *E. coli* O157:H7 to grow during germination and sprouting, and observed that the three experimental pathogens reached approximately 6 $\log_{10}$ CFU/g within 24 h. In a previous study, Castro-Rosas and Escartin (1999) demonstrated that treatment of alfalfa seeds and sprouts contaminated with *Vibrio cholerae* O1 and *Salmonella* Typhi with antimicrobials such as sodium hypochlorite and Citricidal® did not eliminate the pathogens. Thus, alternative methods for postharvest treatment of sprouts are needed.

Ozone exhibits certain characteristics that make it attractive for use as a sanitizer in food processing. It is a strong antimicrobial agent with high reactivity and spontaneous decomposition to a nontoxic product (i.e. oxygen) (Kim *et al.* 1999b). Since ozone decays quickly in water, its use may be considered as a process rather than a food additive, with no safety concerns about consumption of residual ozone in food products. Ozone is used with varied success to inactivate microflora on meat, poultry, eggs, fish, fruits, vegetables, and dry fruits (Kim *et al.* 1999b). The U.S. Food and Drug Administration recently approved the use of ozone in aqueous and gaseous phases as an antimicrobial agent for treatment, storage, and processing of foods (Federal Register 2001).

Ozonation involves the onsite production of ozone gas, from ambient air, by means of an ozone generator. The gas so produced is injected immediately into a water or air stream where it dissolves (Pryor and Rice 1999). Susceptibility of microorganisms to ozone varies with physiological state of the cells, pH of the medium, temperature, humidity and presence of additives such as acids, surfactants and sugars. Relatively low concentrations of ozone and short contact times are sufficient to inactivate pure suspensions of bacteria, molds, yeasts, parasites and viruses (Kim *et al.* 1999b). Ozonation is a nonthermal treatment suitable for easily damaged produce such as sprouts.

Therefore this study was undertaken to determine if aqueous ozone could be used to eliminate *E. coli* O157:H7 present on alfalfa sprouts which may be carried over from seeds to sprouts during the commercial sprout production.

MATERIALS AND METHODS

Preparation of Bacterial Inoculum

Five strains of enterohemorrhagic *E. coli* O157:H7 resistant to nalidixic acid were obtained from the Center for Food Safety, University of Georgia. The strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from an outbreak associated with alfalfa sprouts). Cells were grown in tryptic soy broth (Difco, Sparks, MD) supplemented with 50 μg/mL nalidixic acid (Fisher Scientific, Fair Lawn, NJ) and 0.1% dextrose (TSBN) at 37C for 18 h. The use of nalidixic acid minimized growth of microorganisms other than *E. coli* O157:H7 on enumeration media. A mixture of the five *E. coli* O157:H7 strains was prepared by combining 100 mL of each 18 h culture and centrifuging (Sorvall STH750, Kendro Lab Products, Newtown, Conn.) at 4C and 3,300 $\times$ *g* for 15 min. The supernatant was decanted and the pellet was resuspended in 300 mL of sterile 0.1% peptone (Bacto, Sparks, MD) water before centrifuging again at 3,300 $\times$ *g* for 15 min at 4C. The pellet was then resuspended in 1 L of sterile 0.1% peptone water.

Inoculation of Alfalfa Sprouts

Alfalfa sprouts were obtained from a local grocery store. Four hundred grams of alfalfa sprouts were soaked in 1 L of 0.1% peptone water containing the five strain suspension of *E. coli* O157:H7 ($\sim 10^8$ CFU/mL) for 1 min with gentle agitation. After the inoculum was decanted, sprouts were placed on a sterile perforated tray lined with four layers of cheesecloth and dried in a laminar flow hood at room temperature (21 $\pm$ 1C) for 1 h. Sprouts were stored at 4C in Ziploc® bags for 24 h to allow enough time for attachment of bacterial cells.

To determine the population of *E. coli* O157:H7 on sprouts, 10 g of sprouts were stomached in 40 mL of 0.1% peptone water for 30 s and serially diluted. The stomachate (0.1 mL) was surface plated on tryptic soy agar (Difco, Sparks, MD) supplemented with 50 μg/mL nalidixic acid (TSAN). The plates were incubated for 24 h at 37C before counting presumptive colonies of *E. coli* O157:H7. A few colonies picked from randomly selected plates of various replicates/treatments were confirmed by *E. coli* O157:H7 latex agglutination test (Remel Microbiology Products, Lenexa, Kan.).

Preparation of Ozonated Water

Ozone gas (0.34 m^3/h) was generated using a lab-scale ozone generator (Model No. H-50, Hess Machines International, Ephrata, Pa.) equipped with an oxygen concentrator. Two liters of sterile deionized water at 4C in a 2 L Erlenmeyer flask fitted with a silicon stopper, inlet, and exit lines, was sparged with ozone through a 10 μm stainless steel sparger for 1 h to attain 21 ppm aqueous ozone. Excess ozone was passed through a 2% potassium iodide solution to prevent ozone from being released into the environment. The sparging process was performed in a fume hood for safety purposes. Also the temperature of water during ozonation was maintained at 4C, since the solubility of ozone is higher at lower temperatures (Hill and Rice 1982).

The concentration of ozone in the water was determined by direct measurement of UV absorption at 258 nm as described in an earlier study (Sharma *et al.* 2002).

Treatment of Alfalfa Sprouts with Ozonated Water

The mode of introduction of ozone, ozone availability, and reaction time can influence the rate of cell inactivation. Three different methods for treating sprouts with ozonated water, coupled with various exposure times and pressure, were evaluated for effectiveness in inactivating *E. coli* O157:H7. Each experiment was replicated three times.

Treatment with Water Containing 21 ppm Ozone. Twenty-five grams of contaminated alfalfa sprouts were soaked in 1 L of ozonated water (4C) initially containing 21 ppm ozone. The sprouts were submerged in the ozonated water at 4C for 2, 4, 8, 16, 32, and 64 min. Ozonated water was replaced with sterile deionized water for control treatments using the same temperatures and times.

Continuous Ozone Sparging. Ozone gas was directly sparged into 1 L of sterile deionized water, maintained at 4C, containing 25 g of inoculated sprouts. Sparging times of 2, 4, 8, 16, 32, and 64 min were tested. The control for continuous sparging of ozone consisted of sparging with air instead of ozone. Inoculated sprouts were placed in sterile deionized water at 4C for the same exposure times as those used for sprouts subjected to continuous ozone sparging.

Continuous ozone sparging was followed by 12 psi hydrostatic pressure treatment. A 15.5-quart (17.07 L) pressure sterilizer (Model No. 1915X, Wisconsin Aluminum Foundry Co., Manitowoc, Wisc.) was modified to make a pressure vessel suitable for treating sprouts in ozonated water. Appropriate inlet and exit valves were provided to create and release pressure as desired.

Twenty-five grams of contaminated alfalfa sprouts immersed in 1 L of sterile deionized water (4C) in a 1000 mL beaker were placed in the pressure vessel. Ozone gas was continuously sparged through the water containing the sprouts. After treating the sprouts with ozone for 2, 4, 8, 16, 32, and 64 min, the exit valve of the vessel was closed and pressure was allowed to increase to 12 psi within 3.5 min. Twelve pounds per square inch was the maximum pressure that could be obtained with the flow rate of the ozone gas. Ozone flow was stopped and the pressure vessel was allowed to stand for 5 min before releasing the pressure and analyzing sprouts. Due to the short half life of ozone, a longer holding time was not expected to provide additional benefits. Alfalfa sprouts subjected to the pressurized treatment provided a total treatment time equivalent to the sum of sparging time, come up time for pressure, and holding time. The objective of applying pressure subsequent to continuous ozone sparging was to increase accessibility of ozone into difficult to access areas of sprouts where *E. coli* O157:H7 may be hidden. Continuous ozone sparging without application of pressure served as the control.

Microbiological Analysis

To determine the initial population of *E. coli* O157:H7 on sprouts before treatment with ozone, 10 g of contaminated sprouts were placed in 40 mL of sterile 0.1% peptone water in a Stomacher® 400 bag for 2, 4, 8, 16, 32, and 64 min in order to provide the same soaking time, due to release of bacterial cells from the sprouts. Sprouts were pummeled for 30 s and the wash solution was serially diluted in sterile 0.1% peptone and surface plated (0.1 mL) in duplicate on TSAN. After incubating plates at 37C for 24 h, presumptive *E. coli* O157:H7 colonies were enumerated.

Populations of *E. coli* O157:H7 on ozone-treated sprouts were determined by placing the treated sprouts (25 g) in 100 mL of sterile 0.1% peptone water followed by pummeling in a Stomacher® for 30 s, serially diluting in 0.1% peptone water and surface plating on TSAN. Colonies formed on plates inoculated with peptone wash samples from both untreated and treated sprouts were randomly picked and confirmed by *E. coli* O157:H7 latex agglutination test (Remel Microbiology Products, Lenexa, Kan.).

Effect of Ozone Treatment on Appearance of Alfalfa Sprouts

Alfalfa sprouts treated with ozone and water were visually examined for change in color, general appearance and breakage after treatment in comparison with untreated sprouts.

Statistical Analysis

All treatments were replicated three times. The results were analyzed using the general linear model approach in MINITAB (Minitab Inc., State College, Pa.) for analysis of variance and determining significant and nonsignificant differences in $\log_{10}$ CFU/g of *E. coli* O157:H7 on alfalfa sprouts subjected to each treatment. A 95% confidence level was used for analysis.

RESULTS AND DISCUSSION

Ozone Treatment with Water Containing 21 ppm Ozone

Inoculated alfalfa sprouts were soaked in water containing an initial ozone concentration of 21 ppm for up to 64 min. The concentration of ozone in the water during and after treatment could not be measured with the available method due to biosafety concerns and turbidity caused by release of insoluble sprout components. Analysis of water with initial ozone concentration of 21 ppm in which uncontaminated sprouts were soaked for 64 min did not show any residual ozone. The treatment resulted in population reductions ranging from 0.67 to 0.85 $\log_{10}$ CFU/g (Table 1). The initial population on untreated sprouts was ~7 $\log_{10}$ CFU/g. Lower initial ozone concentrations were not tested because results from a previous study on alfalfa seeds inoculated with *E. coli* O157:H7 did not show significant differences in most treatments at low ozone concentrations (Sharma *et al.* 2002).

The control treatment with sterile deionized water resulted in reductions in population of *E. coli* O157:H7 from 0.63 $\log_{10}$ CFU/g at 2 min to 0.79 $\log_{10}$ CFU/g at 64 min. Analysis of variance using a general linear model involving two way interaction terms ($P \leq 0.05$) showed that reductions with control were not significantly different from those achieved using ozonated water.

Regardless of the type of treatment (sterile deionized or ozonated water), the reductions in population of *E. coli* O157:H7 were not significantly different at contact times of 2 to 64 min. This may be due to the rapid decrease in ozone concentration upon contact with the alfalfa sprouts. Therefore, a system to continuously sparge ozone in the treatment water was evaluated.

Continuous Sparging Treatment

Sprouts were continuously sparged with ozone for 2, 4, 8, 16, 32, and 64 min. Continuous ozone sparging replenished ozone in contact with sprouts throughout each treatment. The residual ozone concentration in the water could not be measured due to release of biohazard debris. However, to estimate the concentration of ozone after each treatment, uncontaminated sprouts in sterile deionized water were sparged with ozone for up to 64 min and the water filtered through 0.2 micron filter before the absorbance measurement. The concentrations of ozone after 2 and 4 min could not be detected due to loss of ozone concentration during filtration, while ozone concentrations after 8, 16, 32, and 64 min were 7.24, 8.85, 9.59, and 9.35 ppm, respectively. The reductions in populations of *E. coli* O157:H7 are listed in Table 2. In general, log reductions increased with time of treatment with ozone. The lowest reduction observed after ozone sparging was 0.83 $\log_{10}$ CFU/g of sprouts treated for 2 min. Continuous ozone sparging of sprouts for longer times gave higher reductions. The 1.25 and 1.27 $\log_{10}$ CFU/g reductions at 4 and 8 min, respectively, were significantly greater ($P \leq 0.05$) than reductions after the 2 min treatment, but significantly lower than reductions after 16 and 32 min treatments. The reduction in population on sprouts treated with ozone for 64 min was 2.20 $\log_{10}$ CFU/g, significantly higher than all other treatments.

TABLE 1.
REDUCTION IN POPULATION OF *E. COLI* O157:H7 ON ALFALFA SPROUTS SOAKED IN WATER AT INITIAL OZONE CONCENTRATION OF 21 ppm FOR UP TO 64 MIN

Contact Time (min)	Population Reduction ($\log_{10}$ CFU/g)[1, 2]	
	Control[3]	Treated Sprouts
2	0.63 (±0.10)	0.70 (±0.04)
4	0.72 (±0.04)	0.81 (±0.03)
8	0.79 (±0.12)	0.80 (±0.23)
16	0.69 (±0.07)	0.67 (±0.13)
32	0.78 (±0.04)	0.82 (±0.09)
64	0.79 (±0.03)	0.85 (±0.02)

[1] Within the same row or column, values are not significantly different.
[2] Values in brackets represent Standard Deviation.
[3] Controls consisted of submerging sprouts in sterile deionized water.

TABLE 2.
REDUCTION IN POPULATION OF *E. COLI* O157:H7 ON ALFALFA SPROUTS TREATED BY CONTINUOUS SPARGING OF OZONE FOR UP TO 64 MIN WITH AND WITHOUT SUBSEQUENT APPLICATION OF PRESSURE

Contact Time (min)	Population Reduction ($\log_{10}$ CFU/g)[1, 2]		
	Air Sparged[3]	Ozone Sparged[4]	Ozone Sparged, then Pressurized[5]
2	B 0.83 (±0.03) A	D 0.83 (±0.09) A, a	E 1.03 (±0.21) a
4	B 0.72 (±0.07) A	C 1.25 (±0.06) B, a	DE 1.22 (±0.19) a
8	B 0.83 (±0.08) A	C 1.27 (±0.04) B, a	CD 1.41 (±0.01) a
16	A 1.14 (±0.04) A	B 1.49 (±0.09) B, a	BC 1.63 (±0.10) a
32	A 1.23 (±0.04) A	B 1.63 (±0.04) B, b	A 2.00 (±0.02) a
64	A 1.29 (±0.11) A	A 2.20 (±0.07) B, a	AB 1.92 (±0.13) a

1 Within the same row, for respective control and treatments, values not followed by the same letter are significantly different ($P < 0.05$). Within the same column, values not preceded by the same letter are significantly different ($P < 0.05$).
2 Values in brackets represent Standard Deviation.
3 Sterilized deionized water continuously sparged with air.
4 Controls consisted of submerging sprouts in sterilized deionized water continuously sparged with air.
5 Controls consisted of submerging sprouts in sterilized deionized water continuously sparged with ozone (no pressurization). Total treatment time was the sum of sparging time, come up time for pressure (3.5 min), and holding time (5 min).

The corresponding control gave population reductions ranging from 0.83 $\log_{10}$ CFU/g at 2 min to 1.29 $\log_{10}$ CFU/g at 64 min. Apart from the 2 min ozone treatment, other ozone sparged treatments were significantly more effective in reducing *E. coli* O157:H7 populations on alfalfa sprouts compared to the controls. The reduction in population of *E. coli* O157:H7 on sprouts held in deionized water for 2 to 8 min were significantly lower than that for 16 to 64 min, probably due to availability of less time to draw out the pathogens hidden in the crevices of sprouts.

Continuous Ozone Sparging Followed by Pressurization of Ozone

Continuous ozone sparging significantly increased the effectiveness of ozone in the inactivation of *E. coli* O157:H7. However, to further enhance the accessibility of ozone into area in the sprouts that may harbor the pathogen, pressurization after ozone treatment was investigated. Sprouts were treated by continuous ozone sparging for up to 64 min and then pressurizing at 12 psi for 5 min. Ozone treatments resulted in maximum reductions of *E. coli* O157:H7 populations up to 2.0 $\log_{10}$ CFU/g (Table 2).

Reductions up to 2.2 $\log_{10}$ CFU/g were obtained in ozonated sprouts that were not subjected to pressure. Analysis of variance exhibited no significant differences between pressurized and nonpressurized treatments except at 32 min, where the reduction for the control was significantly lower than pressurized ozone treatment.

The reductions for both control and pressure treatments exhibited a significant increase with increase in sparging time. The population reduction for pressurized treatment increased from 1.03 $\log_{10}$ CFU/g at 2 min to 1.92 $\log_{10}$ CFU/g at 64 min. Nonpressurized treatments for 2 to 64 min resulted in a population decrease of 0.83 to 2.20 $\log_{10}$ CFU/g.

As mentioned earlier, the concentration of ozone in the water after treatment was not determined. However, to estimate the amount of ozone in the water after treatment, sterile deionized water containing sprouts was continuously sparged with ozone with subsequent pressurization. The concentration of ozone in the filtrate ranged from 7.75 - 17.13 ppm for 2 to 64 min sparging time.

Effect of Ozone Treatment on Quality of Sprouts

Subjective visual examination of control and treated alfalfa sprouts revealed that sprouts treated with ozone appeared whiter and cleaner than the sprouts treated with water alone. This was probably due to the washing effect of water and oxidation of pigments caused by ozone. There was no breakage of cotyledons and hypocotyls in any treatment. With the exception of 64 min treatments with ozonated water which caused shoots to become slightly shrunken, the appearance of sprouts treated with ozone was not substantially different than that of the control sprouts. Thus, it is concluded that for treatment times less than 64 min, ozonated water did not have detrimental effect on visible quality of sprouts.

A certification program instituted by the International Sprout Growers Association, under a voluntary quality assurance program, requires that sprout producers apply a seed disinfection treatment of 20,000 ppm calcium hypochlorite. Use of an alternative treatment is permitted if it reduces the population by more than 3.5 $\log_{10}$ CFU/g and complies with EPA and FDA requirements

(NACMFC 1999b). Of the studies conducted to determine the effectiveness of various chemicals in inactivating pathogens on sprouting seeds, no single treatment demonstrated reductions of more than approximately 3 logs (Taormina *et al.* 1999). In addition, the use of high concentrations of chemicals such as chlorine in treatment solution may result in residues and by products potentially problematic for the environment and human health (Richardson *et al.* 1998).

The natural microflora was reduced by < 1 log when sprouting mung bean was treated with water containing 100 ppm chlorine (Splittstoesser *et al.* 1983). Reduction was 2 logs during treatment of mature sprouts with 5000 ppm chlorine. Jaquette *et al.* (1996) reported that an initial *Salmonella stanley* population of 3.29 $\log_{10}$ CFU/g on dry alfalfa seed increased to 7.08 $\log_{10}$ CFU/g during sprouting, and even pregermination treatment of the seeds by soaking in 2,000 to 4,000 ppm chlorine did not guarantee pathogen free sprouts. Reduction of *Salmonella* Typhi on alfalfa sprouts treated with 200 ppm sodium hypochlorite was less than 1.5 $\log_{10}$ CFU/g (Castro-Rosas and Escartin 1999).

Ozone in the gaseous or aqueous phases was used with mixed success by researchers to inactive a majority of microorganisms (Kim *et al.* 1999b). Inactivation of microorganisms by ozone is greater in ozone demand free systems. In food systems rich in organic matter, ozone reacts with other particles and its effectiveness depends on the residual ozone in the medium. Zhao and Cranston (1995) sparged ozonized air through cell suspensions of *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, *B. cereus*, *Aspergillus* spp., and *Penicillium* spp. They obtained over five log reductions in time periods ranging from 5 to 20 min. Sparging of ozonized air through ground black pepper at various moisture contents reduced microbial populations by 3 to 6 logs. Though it caused oxidation of volatile constituents at longer treatment times, it seemed an attractive alternative to chemical preservatives, if the traditional spice processing could be changed from treating ground spices to washing whole peppercorns. Beyer and Hampson (2001) used ozone at 4 ppm to irrigate broccoli seed sprouts inoculated with wild type *E. coli*. Analysis of sprouts after 6 days of growth resulted in a 1 $\log_{10}$ CFU/mL reduction in *E. coli*. Bubbling of ozone in a mixture of shredded lettuce and water for 5 min reduced mesophilic and psychrotrophic bacteria by 3.9 and 4.6 log, respectively (Kim *et al.* 1999a). Bubbling gaseous ozone in water was the most effective ozonation method.

Reductions in population of *E. coli* O157:H7 on alfalfa sprouts subjected to continuous sparging with water or ozonated water were higher than respective reductions on sprouts not subjected to continuous sparging. This could be attributed to the continuous supply of ozone and simultaneous agitation caused by movement of air or ozone gas in the water. None of the treatments was successful in completely eliminating *E. coli* O157:H7 from the sprouts. Nevertheless a reduction of up to 2.2 $\log_{10}$ CFU/g indicates that ozone treatment offers potential as an alternative to chlorine treatment of seeds to reduce populations of pathogens on alfalfa sprouts. Though improvised methods for facilitating ozone delivery into areas of sprouts that may protect pathogens against contact with ozone need to be investigated, application of hydrostatic pressure remains an alternative to be explored.

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